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Advance in Biological Research, 2024, 5(2), 49-64
https://www.hillpublisher.com/journals/abr/
ISSN Online: 2635-0319

Single-omics Approaches to Improve Abiotic Stress Tolerance in Faba Bean: A Review

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How to cite this paper: Buzuayehu Mamo Mekuria, Tiruwork Zewdie, Deshu Mamo, Adugna Abdi. (2024). Single-omics Approaches to Improve Abiotic Stress Tolerance in Faba Bean: A Review. *Advance in Biological Research*, 5(2), 49-64.
DOI: 10.26855/abr.2024.12.003

Received: January 10, 2025
Accepted: February 7, 2025
Published: March 3, 2025

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Abstract

Faba bean (*Vicia faba L.*) is the fourth most extensively planted cool-season legume, following pea, chickpea, and lentil. It contains more protein than other common dietary beans used for food and feeds around the world. Faba beans are highly susceptible to abiotic stresses such as drought, frost, heat, and salt, which significantly reduce both crop growth and yield. For instance, drought alone can reduce faba bean yields by up to 50%, during early pod-setting, highlighting the urgent need for strategies to enhance. The absence of a fully annotated reference genome for faba beans complicates the use of CRISPR/Cas gene editing techniques, which rely on detailed genetic maps to precisely target genes involved in stress tolerance. Developing such a genome could significantly enhance the effectiveness of gene-editing strategies. Proteomic approaches were utilised to identify a variety of abiotic stressors, including heat, drought, salt and frost. Proteomic analysis reveals that a significant portion of the differentially expressed proteins in faba beans are involved in essential processes such as photosynthesis, glucose metabolism, and stress defense pathways. These proteins play crucial roles in mitigating the negative effects of drought and heat stress, contributing to improved stress tolerance in the plant phenotype. Metabolomics has been used successfully in a variety of faba bean stress studies, including metabolic pathway studies, genotype-biochemical phenotype correlations, silent phenotype mutations, plant-environment interactions, and plant priming, a phenomenon that prepares plants for enhanced stress defence. Metabolites, such as proline and sugars, are directly involved in stress tolerance mechanisms and exhibit a stronger correlation with the plant's phenotype than mRNA or proteins. For example, increased levels of proline under drought conditions help in osmoregulation, a key trait for enhanced drought tolerance. Although all mineral nutrients and trace elements are required for plant growth and development. Various OMICs approaches can be used to target the faba bean abiotic stress responses generated by genomic sequences, transcripts, protein organisation and interactions. In general, this review concentrated on the faba bean genomics, transcriptomics, proteomics, metabolomics, phenomics and ionomics under abiotic stress.

Keywords

Abiotic stress; Faba bean; Genomics; Ionomics; Phenomics; Proteomics; Metabolomics; Transcriptomics

1. Introduction

The faba bean (*Vicia faba L.*), one of the first domesticated edible legumes, has a lengthy agricultural history, with seeds dating back 14,000 years [1]. Faba bean is the fourth most commonly planted cool-season grain legume (pulse) globally, behind pea (*Pisum sativum L.),* chickpea (*Cicer arietinum L.*), and lentil (*Lens culinaris Medik.*), with an annual yield of around 4.5 million tonnes from close to 2.5 Mega hectar [2]. It is widely farmed for food, and feed as a source of high-quality protein, dietary fibre, and other vital ingredients [3]. Faba bean generates proteins that are safe to consume by anyone as food. Its grain contains a protein content of approximately 24%-30% [1, 4], of their dry matter, making them a major source of affordable protein for people in the Middle East, and Latin America, and Africa. It is regarded as an excellent protein crop due to its ability to supply nitrogen inputs into temperate agricultural systems and its high yield potential when compared to all grain legumes. Moreover, like most other legumes, it develops a symbiotic relationship with nitrogen-fixing nodule-forming bacteria, which gives significant benefits to cropping systems and the environment while also contributing to agricultural sustainability through soil improvement [4].

The production of faba beans has remained low when compared to other pulse crops, despite its many benefits, including availability and high-yielding cultivars (>3 tons/hectar) in high demand [1]. This is because they are susceptible to a variety of biotic and abiotic issues [5]. Abiotic stressors that drastically lower crop growth and output, like drought, cold, heat, and salt, can affect faba beans. For example, during early pod-setting, drought alone can reduce faba bean yields by up to 50%, underscoring the critical need for methods to increase tolerance [6].

Plants acquired morphological and physiological adaptations, as well as signalling pathways that evoke biochemical and molecular mechanisms, to cope with these extreme climatic conditions [7]. The dynamic responses of plants to diverse abiotic challenges have been extensively studied [8]. Numerous studies have demonstrated that the faba bean can withstand cold and frost [9], water stress and drought [10].

A substantial genetic diversity in faba bean accessions has been documented for numerous drought adaptation features [11, 12]. Other legume crops have had considerable success with drought resistance selection based on physiological properties such as proline accumulation or soluble sugar build-up and highly heritable secondary traits [13]. Substantial variances in faba bean genotypes under different water regimes were discovered as the best option [14].

In recent decades, several intriguing omics technologies have emerged. These omics-based approaches have proven effective for exploring the genetic and molecular foundation of crop development through changes in DNA (genomics), transcript levels (transcriptomics), proteins (proteomics), metabolites (metabolomics), and (ionomics) mineral nutrients in response to environmental and physiological stress [15].

Advances in genomic research have led to the identification of various gene families and pathways that regulate tolerance to abiotic stressors [16]. These genes may be associated with protein kinases such as mitogen-activated or calcium-reliant kinases, which in turn activate transcription factors and cis-acting elements that govern stress-response patterns. Transcriptomics is a potential method that are responsible for abiotic stress resilience in legumes and aid in crop breeding by identifies the gene correctly through long read [17, 18]. Proteomics is a relatively recent approach for discovering proteins and pathways associated with plant stress response and physiology [15]. It is possible to strengthen this technique by protein concentration, and/or enzyme activities, thus generating metabolite correlation networks. Metabolites have a closer relationship to the phenotype than mRNA or proteins because they represent gene expression and interactions that affect gene regulation under stress conditions [19].

Ions have a substantial role in the maintenance of a plant's homeostasis under different environmental conditions. It takes considerable knowledge of the gene regulatory networks involved in ion homeostasis to use ionomics to evaluate ion transporters, genes, ions, and elements that are responsive to abiotic stress [20]. Omics technology not only expands our understanding but also gives new light on how stressed plants behave [21]. Also, it is a rapidly expanding technology that enables to increase awareness and identification of all genomic and transcriptomic processes in plants [18, 22]. Thus, the objectives of this review were to describe the application of single omics (Genomics, Transcriptomics, Proteomics, Metabolomics, Phenomics and Ionomics) for faba bean under abiotic stress, analysis tools used in single omics, and challenges and prospects as problem-solving agricultural biotechnology tools for faba bean.

2. Single-Omics Approaches for Abiotic Stress

A successful expression of genomics, transcriptomics, proteomics, metabolomics, phonemics, and ionomics, breeders will be aided in identifying promising candidate genes and ideal features for producing and improving the productivity of legume crops under abiotic stresses [23]. Genomics can help to elucidate genetic variance, which can improve crop

breeding efficiency and lead to crop species genetic improvement [24]. Transcriptomics is a powerful tool for assessing gene expression as well as obtaining an accurate image of a target cell or tissue. Proteomics serves as a link between its transcriptome and metabolome, showing the true status of its biological response more accurately than DNA markers. Because proteins typically undergo post-translational modifications that influence their true function, the transcriptome's cellular mRNA levels do not adequately describe protein expression. These proteins are involved in systems for stress adaptation and repair, as well as signal transduction pathways. They allow the plant to recover from a stress injury and, as a result, survive under extreme stressors.

Metabolomics can be used to determine how organisms respond to their environment at the metabolite level [25]. Abiotic stress considerably alters plant metabolomes and proteomes because they actively participate in defence mechanisms against diverse stresses [26]. It is a good tool for understanding the changes in plant metabolism that occur as a result of contact with the outside environment [23]. The phenotype of an organism is the result of a complex interaction between its environment and genetic potential [27]. Ionomics can bridge the gap between the understanding of a genotype and the phenotype it regulates when paired with other high-throughput technologies such as proteomics, transcriptomics, and metabolomics [18]. The next paragraph of this review was mainly focused on faba bean genomes, transcriptomics, proteomics, metabolomics, phenomics and ionomics for abiotic stress responses, challenges and future prospects.

2.1 Genomics

The genomics approach offers a comprehensive view of gene structure and functional analyses, as well as the eidentification of genetic variants that can be employed to overcome abiotic stress in plants. It focuses on the physical integrity of the genome, intending to detect, diagnose, and regulate genomic features throughout the chromosomes [18]. Structural genomics includes sequence polymorphism and chromosomal organisation and allows plant researchers to create physical and genetic maps to find features of interest. In contrast, functional genomics sheds light on the roles of genes in the regulation of the trait of interest. Epigenomics refers to the phenomena of epigenetic alterations occurring at the genomic level in the form of histone modifications, DNA, or small RNA methylations. Pangenomics, is defined as the sum of a core genome shared by all persons and a dispensable genome that is either partially shared or individual specific. Mutagenomics and pangenomics are contemporary omics techniques in agricultural sciences that focus on mutagenesis and the pangenome, respectively [24].

Faba bean progress is now hampered because of the creation of rich genetic resources has lagged behind those of other cool-season grain legumes. Faba bean is a largely allogamous diploid species with six pairs of chromosomes. It has one of the biggest genomes of any diploid field crop, with around 13 Gbp in the haploid complement and more than 85% repetitive DNA [3. The genome of the faba bean is 2.6, 3.2, and 17.6 times larger than the genomes of the pea, lentil, and chickpea, respectively [3]. Faba bean genome assembly and map-based cloning were both delayed due to its genome complexity (e.g., the abundance of transposable elements) and its status as a minor crop. Furthermore, the development of high-density genetic maps obtained from numerous populations, as well as gene-based molecular markers has paved the way for marker-assisted selection (MAS) and gene discovery [28].

Despite the faba bean's vast genome, research into the genes that underlie cold tolerance has been hampered by a lack of genomic data [18]. Genomic approaches are now being employed in the faba bean giving new opportunities for fine mapping and finding candidate genes [3]. SNP-based DNA markers in faba bean have made it easier to generate highly saturated and cost-effective second-generation genetic maps. The high-density faba bean genotyping array was recently developed [3, 29]. It has 24929 polymorphic high-resolution SNP markers in 15846 different genes. SNP markers are thus recognised as useful tools for faba bean genetic mapping, association studies, identifying genetic diversity, and positional cloning [28]. The large faba bean genome is now being built to advance faba bean genomics and breeding [30].

2.1.1 Genomic map

In faba bean, genetic linkage maps were created utilising various populations and molecular markers was the first to publish a genetic map of the faba bean using just 17 markers, discovering 19 genetic variables that created four linkage groupings [3]. In the 1990s, morphological markers, isozymes, seed protein genes, and random amplified polymorphic DNA (RAPD) markers were used to conduct genetic mapping research. Later, the development of ESTs, microsatellites or single sequence repeats (SSRs), and single nucleotide polymorphism (SNP) markers aided in the enrichment of faba bean genetic studies and breeding. A database of faba bean ESTs, mtSSRs (mitochondrial-simple sequence repeats), and microRNA-target markers was recently launched [31].

The first *V. faba* genomic map was created by [32], dividing all markers into six linkage groups that corresponded to

the six haploid chromosomes. This work created a map with just 687 markers dispersed across 1,403.8 cM in six linkage groups, each of which was attributed to a physical chromosome [33]. It allowed all gaps in marker coverage to be quantified, and users of the consensus map could choose an individual trait-linked marker and easily replicate it, or take a subset of spaced markers to sample the entire genome or simply use the entire set. Importantly, the 687 mapped markers included 34 converted CAPS markers from prior research, implying that the new consensus map was backwards compatible [32]. As a result, SNP genotyping products with orders of magnitude higher density are now possible.

2.1.2 Trait mapping for abiotic stress tolerance in faba beans

Frost resistance is a key breeding focus for increasing production stability by overcoming plant abiotic stress [12]. The Quantitative trait loci (QTL) analysis is a very useful tool for predicting potential/putative genes and their roles. Frost tolerance is a highly heritable trait with strong additive effects that are influenced by a large number of genes or quantitative trait loci. Furthermore, the effectiveness of selection was hampered by genotype-environment interaction [34]. Given the difficulties mentioned above, marker-assisted selection opens the door to dissecting frost tolerance at the genetic level. Validation can be checked using multiple genetic backgrounds and population creation methods [for example, backcross, recombinant inbred lines, and multi-parent advanced generation inter-cross (MAGIC) population]. Key putative QTL for frost tolerance and fatty acid composition (FAC) that relieve the effect of frost stress in faba bean leaves at the seedling stage have been identified [2].

Roots play an important role in plant growth during drought stress. Despite the fact that the faba bean's response to drought stress has been widely studied [35], few molecular approaches have been applied to boost drought tolerance in this crop. The candidate genes inside the QTLs for stomatal characteristics on chromosomes were identified [3].

2.1.3 Genome-wide association study for faba bean abiotic stress tolerance

GWAS analysis has recently gained popularity [36]. Because of its ability to discover genes, genomic sites, and genomes that are associated with beneficial crop qualities. It has become an essential approach for identifying the genetic variants underpinning complex traits during drought stress. The large number of SNPs identified using Affymetrix's 50K SNP array [3, 29] has provided researchers with full genome coverage, allowing them to differentiate between different germplasm accessions and conduct high-resolution association mapping. 52 significant SNPs were discovered across the six faba bean chromosomes using 21,915 SNPs, which combined accounted for a considerable percentage of the total phenotypic variance, and 29 significant SNPs under drought conditions [13]. Several genes or QTL govern the faba bean's resilience to low temperatures. Previous faba bean research using SNP and AFLP markers in multi-parent advanced generation inter-cross populations discovered QTL associated with cold tolerance [37].

2.1.4 CRISPR for abiotic stress tolerance

In recent decades, the development of crop plants that can withstand abiotic stress has been transformed by genome-editing technology, which is regarded as an important instrument. CRISPR-associated protein 9 (Cas9) and a single guide RNA (sgRNA) make up the two-component CRISPR/Cas9 system. The protospacer-matching CRISPR RNA (crRNA) and the transactivating crRNA are two distinct RNAs required for CRISPR activity that are synthesized into the sgRNA. As part of the sgRNA/Cas9 complex, the 20 nucleotides at the 5' end of a sgRNA attach to the target genomic location. A blunt-ended double-strand break (DSB) is caused by the cleavage of target DNA in the genome by the SpCas9 protein, a large (1368 amino acid) multi-domain DNA endonuclease. Ultimately, the host cellular machinery fixes the DSB. Nowadays, the genetic mechanism behind tolerance against a variety of abiotic stimuli, such as drought, salt, heat, and nutritional values in different agricultural plants, has been successfully understood by CRISPR/Cas-based genome engineering [23].

The selection of an appropriate promoter for cas9 expression, the design of guide RNA, the creation of new alleles for abiotic stress-responsive genes, and the creation of an appropriate gene delivery system are the primary considerations in the CRISPR/cas9 gene-editing system for enhancing abiotic stress resistance in plants [38]. In this case, the best targets for crop development are known positive and negative regulators of a certain characteristic. By altering biochemical pathways, metabolite profiles, and physiology, knockouts in structural genes, regulatory genes, transcription factors, and promoter regions result in altered and broken protein products and create stress-tolerant phenotypes. Reduced plant biomass, photosynthetic rate, SOD, CAT, GPX, and PAL activities, as well as chlorophyll content and increased reactive oxygen species (ROS), flower and pod abortion, transpiration rate, ion leakage, and lipid peroxidation, are all caused by this stress-induced expression of the abiotic stress-responsive gene [23].

The faba bean genomics and breeding revolution will be accelerated. Despite the lack of a reference genome for the faba bean, great progress has been made in the establishment of genetic and genomic resources to facilitate molecular breeding.

The absence of a fully annotated reference genome for the complex faba bean genome impedes the application of CRISPR/Cas gene editing, which relies on detailed genetic maps to precisely target genes involved in stress tolerance. There has been no CRISPR/Cas9 research for this crop to date [18]. So that developing such a genome could significantly enhance the effectiveness of gene-editing strategies.

Table 1. Summary of published Genome data in faba bean

Abiotic stress	Tissue	Marker type	Chromosomes/region/gene/traits	References
Frost	Leave	67 putative QTL	alleles increasing unsaturated fatty acid content and proline	[3]
Frost	Leaves	117 SNP markers	Chromosome 2	[34
Physiological traits and frost tolerance	Leaf fatty acid	17 QTL	Chromosome 5	[34]
Frost/cold	Root	5 SNPs	chromosomes 3 and 5	[34]
Frost tolerance	seedlings, flowers, and pods	Accession (11NF003a-11, and 11NF010a2)	Chromosome 3 IX474/4-Warda,	[2]
Frost tolerance and physiologically related traits	Leave	131 RAPDs, 1 morphological	F6, 1635 map length (cM)	[3]
Heat tolerances	Leaves	SNPs (10,749)	Chromosome 9 and 11	[9]
Drought adaptation-related and morphological traits	Leave	188 SNP markers, 1 morphological	F5, 928 map length (cM)	[3]
Drought-tolerant genotypes	Seed	Nubaria-2, and Nubaria-3,	Chromosome 1 and 2	[13, 10]

2.1.5 Genomic analysis tools

The use of electrophoresis and purification systems to isolate DNA templates, PCR and sequencing to determine the sequence and map of the DNA base code, microarrays and genotyping to determine sequence similarity and differences and next-generation sequencers to analyse whole genomes [3].

2.1.6 Genomics: challenges and prospects

Recent scientific advancements have made it possible to sequence the full faba bean genome. Saturated synteny-based genetic maps, NGS, and high-throughput genotyping technologies for Faba bean are now accessible, significantly improving genome assembly. Wide genome sequencing efforts, however, have been limited primarily by (1) lengthy and repetitive cereal genome sequences and (2) a lack of modern technology and algorithms capable of producing and assembling large and correct sequences [39]. As a result, this was most certainly the most crucial cause for the need for large international consortia activities. Several collaborative reference genome assembly projects in faba beans are currently underway. The absence of an annotated reference genome for the complex faba bean genome stymies CRISPR/Cas gene editing, especially when creating specific gRNA-targeted genes of interest. The eventual biological effect on DNA is difficult to anticipate due to epigenetics, post-transcriptional, and post-translational modifications [40]. Although large-scale genome sequence production and assembly are now expensive, future large-scale, reference-quality genome assemblies will be easier due to the low cost-outcome differential.

It is reasonable to expect that genome assembly will continue until the difference between whole-genome genotyping and whole-genome sequencing is minor. Grain geneticists and breeders will benefit from advances in sequencing and computing capabilities, which will boost output per unit of input. Genome advancements will aid in the quick and accurate mapping of traits. Furthermore, as dense marker information becomes accessible, methodologies for forecasting genotypic or breeding value will become more efficient, resulting in higher genetic gains per unit of labour and money [39].

2.2 Transcriptomics

Several transcriptomes have been reported for faba bean albeit in the absence of a reference genome [41, 42]. These

datasets were generated from a selection of different genotypes and tissues at various development stages or treatments, the transcriptome data coverage has been further enriched. The sequence length data were increased at 461 chromosomal loci and provided increased accuracy compared to transcriptome data [32]. The transcriptome data revealed that faba bean, despite its large complex genome, compared similarly to other legume species in expressed gene content [43].

Enhancing the faba bean's stress tolerance has become important since more harsh climatic circumstances are predicted to arise from climate change. The genomic cascades involved in stress can be swiftly gained through transcriptome profiling, particularly with long reads. The genes encoding heat shock proteins, dehydrins, and late embryogenesis abundant proteins were among the many stress-related genes annotated [44]. The gene expression of phenylalanine ammonia-lyase, anthocyanin synthase, and NADP-D-sorbitol-6-phosphate dehydrogenase was consistent with the transcriptome profile [15].

2.2.1 Transcriptomics response for abiotic stress regulation

Transcriptomics response for drought stress regulation: the extensive range of physiological, metabolic, and cellular processes influenced by drought stress, and the drought-responsive genes in faba bean were identified [33]. Drought stress-responsive differentially expressed genes coded for a variety of regulatory proteins such as protein kinases and phosphatases, transcription factors and plant hormones, and functional proteins such as osmoprotectant, detoxification, and transporter enzymes, the majority of which were largely up-regulated for drought stress in the faba bean [41].

Frost is a significant abiotic factor reducing faba bean output. Genes that respond to frost/cold stress were connected to unigenes (WCOR413, DHN2, HAV22, CBF1-3, ICE1-2, and COR15a-b), as well as unigenes connected to the ICE-CBF-COR pathway [34]. Some of these genes are cold-regulated genes that encode cryoprotective proteins, whereas others encode transcription factors (TFs) or positive regulators of TFs [37] (Lyu *et al.,* 2021). For instance, the ICE gene encodes a bHLH transcriptional activator that resembles MYC [45].

Table 2. Summary of published transcriptome data in faba bean

Abiotic stress	Tissue	Output	NGS platforms	References
Salinity stress	Cotyledon	1410 responsive genes	Illumina HiSeq 4000	[46]
Salinity stress	Seed	4,486 differentially expressed genes	RNA-seq, Illumina Hiseq 4000	[47]
Drought stress	Embryos	5,000, EST differentially expressed genes	GBS 100,037,292 bp.	[48]
Drought stress	Root at vegetative and flowering stages	18,327 SSRs differentially expressed genes	RNA-seq, Illumina Hiseq 4000	[41]
Drought stress	Leaves	A total of 538 and 642 putative TFs,	Illumina HiSeq 4000	[46, 33]
		35 drought related ESTs	Ion channels, kinases, energy production, TFs	[19]
Drought stress	Root	Novel DEGs that showed a change in Expression	Illumina HiSeq 4000	[46]
Drought stress	Vegetative and flowering stages	12,805 up-regulated and 22,338 down-regulated unigenes	RNA sequencing.	[33]
Frost stress	Leaf	genes that encode cryoprotective proteins	unigenes (3597) by isoforms	[37]

2.2.2 Transcriptomics analysis tools

a. Serial analysis of gene expression (SAGE) and microarrays

To accurately depict the level of gene expression in a specific cell or tissue, a potent tool was transcriptomics. Transcriptomics can pinpoint the gene regulatory networks and potential genes implicated in the emergence of the abiotic stress response for breeding. In-depth transcriptome databanks may now be derived using SAGE and microarrays for the development of high-throughput technologies [46].

b. RNA-seq analysis

Data from ribonucleic acid sequencing (RNA-seq) can be used to identify genes that express themselves differently from one another [18]. A large number of transcriptome data can be analysed using RNA-seq analysis, a high-throughput, low-cost sequencing technique. This approach has some advantages over microarray technology, including the ability to identify novel transcripts and does not require genetic information for the construction of probe sets. High-resolution gene expression atlases are created using the RNA-seq or microarray data derived from transcriptome analyses of different crops [46]. They are a helpful tool for studying the expression of genes and proteins involved in abiotic stress response in soybean, chickpea, faba bean, and pea.

c. Sequencing of isoforms

Isoform sequencing (Iso-seq) produces longer reads, which can result in complex assemblies with fully, or partially closed gaps, low structural mistakes, and precise gene annotations [37]. As a result, iso-seq has been employed to do large-scale transcriptome investigations of important crops with big and small genomes [40].

d. Next-generation sequencing (NGS)

NGS-based transcriptome analysis is reasonably cheap and yields enough information to explain single-nucleotide changes, transcript rearrangements, transcriptional and post-transcriptional gene regulation [49]. It is useful for non-model crops that do not have a reference genome. Moreover, NGS techniques are one of the most powerful tools for transcriptome profiling currently available, which have enhanced the efficiency and speed of gene discovery in faba bean. Data from four genotypes (Hedin, Hiverna, 153b, and 2378) were used to construct a high-quality reference transcriptome [50], which includes information from both shoot and root tissues [51].

e. Expressed sequence tags (ESTs)

The first significant contribution to faba bean transcriptome knowledge was the release of around 5,000 EST from developing embryos of the faba bean variety [48]. An Illumina-sequenced library of mixed tissues enriched with embryo transfer cells provided the most comprehensive transcriptome coverage. Drought altered the expression levels of VfDHN4, APETALA2, VfHSP18, and VfAQP2 genes in a transcriptome analysis employing real-time reverse transcriptase-polymerase chain reaction [36].

2.2.3 Transcriptomics: challenges and prospects

The microarray can only reveal the levels of expression of known genes [40]. This was overcome by RNA-seq, which provides an accurate profile of all transcripts present in an organism at any stage or time. However, processing NGS data with RNA-seq takes time because read coverage may not be constant across the genome due to nucleotide content changes between genomic regions. Moreover, posttranslational changes can affect protein expression; the transcriptome should be viewed as an intermediary step. In RNA-seq, a long transcript is predicted to have more reads than a short transcript at the same expression level [39]. RPKM (Reads Per Kilobase per Million mapped reads) or the related FPKM (Fragments Per Kilobase per Million mapped reads), are used to normalise the counts with relation to transcript length and some software programmes that represent RNA-seq data by transformed quantities. Another method is digital gene expression, a recently established technique for statistically assessing gene expression [18].

2.3 Proteomics

Proteomics refers to the study of the quantitative and qualitative expression of proteins under various situations. It can be used to study how genomic regions influence grain protein composition, enzyme participation, and gene expression in different growing circumstances. Proteome profiles can be compared to assess the role of certain proteins in biotic and abiotic stress signalling, as well as stress-resistant proteins that are differentially expressed [52]. Proteins are directly engaged in plant stress response, proteomics research can significantly help to understand the potential relationships between protein abundance and plant stress acclimation. Significant changes in the plant proteome occur as a result of stress-responsive pathways being activated at numerous molecular levels as a result of unfavourable environmental conditions [53]. Proteins perform a wide range of functions. They act as enzymes that catalyse changes in metabolites, and transcriptional factors, serve as protective mechanisms, transport energy or scavenge free radicals, and interact with other molecules. Plants may accumulate or enhance the production of certain proteins with defensive effects depending on the genotype under the study's level of tolerance [15].

2.3.1 Proteomics application for abiotic stress regulations

Drought stress regulation: changes in proteome expression have been found in legume crops such as chickpeas, soybean, faba bean and mungbean during drought stress [53]. Drought stress is widespread throughout the seedling stage, particularly in the early stages, and it harms faba bean growth and yields worldwide. Matrix-aided laser desorption ionization-tandem time-of-flight (MALDI-TOF/TOF) effectively identified 30 proteins, 25 of which were down-regulated and five of which were up-regulated, using 2-DE to identify over 300 proteins. 2-DE using the isoelectric point and molecular weight of different characteristics to separate thousands of proteins is a very good method [54].

Chitinase levels were found to be higher during drought, showing that it was an important component of the plant defense system, possibly having a role in stress tolerance [53]. Drought increased the expression of the 50S ribosomal protein, implying that it protects plants from stress by re-establishing normal protein conformations. Drought also increased the amount of proteins involved in protein synthesis, such as chitinase, Bet protein, and glutamate-glyoxylate aminotransferase [54].

Protein kinases (PKs) are an important class of differentially expressed regulatory proteins, which include calcium-dependent protein kinase, histidine kinases, mitogen-activated protein kinase and phospholipases such as protein phosphatase 2C and serine/threonine-protein phosphatase 2A. PKs are sensor response genes that initiate phosphorylation cascades and so have a considerable impact on how plants respond to drought stress [55]. Proline is another type of osmotic regulatory protein, expected to have a range of roles in plant cells, including osmotic adjustment and subcellular structural stabilization [56].

Salinity affects different physiological and metabolic systems by exerting osmotic and ionic toxicity, in addition to phenotypic alterations, depending on the degree and duration of the stress [57]. Osmotic stress diminishes root system water absorption capacity while increasing water loss from leaves in leguminous crops such as peas, soybean and faba bean [58]. Membrane disruption, nutritional imbalance, the diminished ability of ROS, detoxification, variations in antioxidant enzymes, lower photosynthetic activity, and reduced stomatal aperture are all key physiological alterations caused by osmotic stress.

Table 3. Summary of published Proteomics data in faba bean

Abiotic stress	Tissue	Responses stress	References
Frost tolerance	seedlings	Mt2g027240, serine/threonine kinase family protein, which plays a critical role in the regulation of lipid metabolic	[34]
Drought	Seedlings, leaves	Proteins including chitinase, Bet, and glutamate–glyoxylate aminotransferase, which is used to upregulate leaves under drought stress.	[18]
Drought stress	Leaf and root	photosynthesis, protein metabolism, antioxidant and cell defence mechanisms	[15]
Salt stress	Root	physio-biochemical and molecular changes, regulated by salt stress	[15]

2.3.2 Proteomics tools

A combination of two-dimensional gel electrophoresis, liquid chromatography, mass spectrometry (MS) identification of proteins and matrix-aided laser desorption ionization-tandem time-of-flight (MALDI-TOF/TOF) was used to investigate the proteome under abiotic stress [59].

2.3.3 Proteomics: challenges and prospects

Protein research is complicated due to posttranslational changes. Technique Proteomics enables the detection of alterations in previously unknown and unexpected proteins. Proteomic analysis, which complements transcriptomics and metabolomics in revealing plant cellular pathways, is an important tool for crop development [39]. Recent advances in proteomics technology have allowed us to comprehend plant biology. However, must overcome the limitations of these strategies to build smart crops with high grain quality and resistance to a range of shocks. Emerging techniques including peptidomics, phosphoproteomics, and redox proteomics will provide detailed information regarding molecular interactions and protein function [60].

2.4 Metabolomics

Metabolomics is a potential tool for evaluating stress responses across plant species since primary metabolite structures

are universal and specialized metabolite structures are usually conserved [61]. As a result of technological advancements in mass spectrometry and spectroscopy, highly complex data are being generated. Plants contain almost 250,000 metabolites, with the concentration and total number being much higher in stressed than non-stressed situations. The identification of valid metabolomic markers will improve plant stress tolerance [22].

Metabolites are classified into two categories. Amino acids, carbohydrates, and lipids are examples of primary metabolites with highly conserved structures. Although plant samples are estimated to contain about 200,000 molecules, current metabolomics techniques can only identify about 14,000 plant metabolites [62]. These compounds help to keep proteins and cell membranes stable while also keeping cells turgor. During cold stress, it is easier to find cryoprotective compounds including soluble sugars, sugar alcohols, and nitrogen-containing molecules. This helps plants adapt to freezing conditions by preventing ice from attaching to the plasma membrane and causing cell harm. Secondary metabolites are necessary for plant growth and development. Terpenoids, alkaloids, and polyphenols are more diverse and vary greatly amongst plant species. Secondary metabolites produce a wide range of chemicals that are thought to be important in a variety of biochemical and biophysical processes that occur in plant cells and tissues [63]. These natural compounds' concentrations are carefully controlled by developmental stage, environmental conditions, and adaption processes [61].

2.4.1 Linking metabolomics to phenotype responses

Numerous researchers have observed metabolic patterns in plants under stress conditions. Metabolites can be identified and measured systematically to determine an organism's metabolic response to various conditions as well as the chemical signature of a phenotype.

Abiotic stress alters plant metabolism in a variety of ways. Such as: the synthesis of proline, changes in enzyme activity, an increased need for certain metabolites, an increase in reactive oxygen species levels, low photosynthetic capacity, reduced growth, decreased fertility, and reproductive cessation [61]. Moreover, other metabolites that may affect plants include organic acids, sugars, polyols, tertiary sulfonium, quaternary ammonium molecules, and phenolic compounds [64]. The majority of research has supported amino acids' potential to operate as osmoprotectants [61]. The abiotic stress factors (drought, heat stress and salt) were described in the following paragraphs based on current metabolomics investigations [56].

Drought stress, when water is scarce, physiological mechanisms tend to restrict water loss and enhance water absorption, affecting metabolism [63]. As a result, one of the biological processes involved in maintaining cell turgor is the accumulation of osmoregulators such as sugars, ethanol, polyamines, and amino acids, specifically proline [56].

Proline is the most abundant amino acid in plant tissues under stress [6]. Proline accumulation in plants lowers the deleterious effects of ions on enzyme performance as well as stress-induced free radical production. Drought stress increased the amount of free proline in leaves [65]. Significant genetic heterogeneity was observed in the genotypes studied under osmotic stress and drought conditions, most likely due to a disruption in the water flow from the xylem to the surrounding renal cells [66]. Under ideal conditions, the concentration of proline in faba beans increased as the level of stress increased, and there was no variation in proline concentrations at the genotypic level. Proline accumulation in faba beans also serves as a physiological status indicator, showing whether or not the plant is drought-stressed [6, 11].

Low temperatures and Al^{3+} toxicity restrict root development, salinity and drought have similar impacts on osmotic equilibrium, and cold inhibits both water transport within roots and the plant's ability to create symbiotic relationships [67]. Al^{3+}-drought interactions have been examined in both common beans and soybean [63]. Crops are affected by the interplay of drought and other stresses, rendering them difficult to recover. Because they influenced the transport of photosynthates and oxygen to the nodule and dryness inhibited symbiotic nitrogen fixation [11].

The majority of plant metabolomics research has focused on aerial components, particularly leaves, which are the sections of the plant most affected by drought stress. During dehydration, the aerial component of this species gathered amino acids and polyamines in a manner that was dependent on ABA as well as raffinose, which was synthesised independently of this hormone [68].

Chlorophyll is a major pigment that participates in photosynthesis in plant chloroplasts. Faba bean plants under drought stress grew slowly and had less chlorophyll in their leaves. The primary biological and biochemical activity of D-alanyl-D-alanine-endopeptidase is the biosynthesis of secondary metabolites carotenoids to reduce the reactive oxygen species, as well as the pigment chlorophyll to protect against drought damage [61]. A few significant pathways linked to drought stress that were present in all combinations studied were "plant hormone signal transduction, flavonoid biosynthesis, oxidative phosphorylation, fatty acid biosynthesis, ABC transporters, and biosynthesis of other secondary metabolites.

Heat stress affects leaf metabolomics. A drop in pyruvate can have an impact on the synthesis of phenylalanine,

tryptophan, tyrosine, isoleucine, and alanine [69]. Recent metabolomic study of 3-day heat stress significantly altered the chemical profile of leaves and triggered a long-term plant stress response. The generation of cytokinins, fatty acid metabolism, flavonoids, and terpenes were the most important activities linked to the response to heat stress. Despite the noticed metabolic changes, pollen seems to be more resistant to heat stress than tissues [26].

Salt: Two faba bean types were subjected to low and high salt levels of NaCl, Na_2SO_4, and KCl treatments [70]. Higher Na^+ and Cl^- concentrations are harmful to photosynthesis and cause developmental disruption in plants, which is associated with the osmotic imbalance and toxic ionic effects. *V.faba,* a glycophyte, is a species that is moderately salt sensitive. Both Na^+ and Cl ions are toxic to Faba beans [70]. Several metabolic responses were revealed to be particularly related to the presence of Na^+, K^+, or Cl^-. In conclusion, it needs further studies on faba bean-based metabolic approaches to control abiotic stress, such as Soil acidity, lodging, and cold stress.

Table 4. Summary of published metabolomics data in faba bean

Abiotic stress	Tissue	Metabolites change(s)	responses to stress	References
Salinity	Leaves and roots	Amino acids	myo-inositol, amino acids synthesis carbohydrate metabolism	[18]
		Glycolysis metabolites	Osmoprotectant, energy metabolism	[70]
Drought and heat	Leaves	TCA cycle metabolites	Energy metabolism, nitrogen cycle, phosphorus acquisition, secondary metabolism	[49]
		Sugar	Osmoprotectant	
drought, salinity, osmotic	Leaves	Amino acids	Protein stabilization, antioxidant activity, osmoprotectant, signaling	[33]
		Amino acids	Proline, glycine-betaine, and other compatible osmolytes	[71]
		Peptides	Phytochelatins and metallothioneins	[71]
Heavy metal intoxication	Root and chlorophyll production	Amino acids	Osmoprotectant, phytochelatins synthesis, polyamines synthesis	[72]
		Phenolics, flavonoids, phytochelatin	Antioxidant, ROS scavenging, structural integrity	

Notes. TCA; Tricarboxylic acid, ROS; Reactive oxygen species.

2.4.2 Metabolomics analysis tool

The most advanced method of analysis in metabolomics is targeted analysis [6]. Combination of gas chromatography (GC), liquid chromatography (LC), mass spectrometry (MS), high-performance liquid chromatography (HPLC) in conjunction with other metabolomic approaches have helped to clarify stress tolerance processes and metabolite profiling. It is possible to precisely determine the concentration of a small number of known metabolites. Contrary to high throughput methods for protein, RNA, and DNA analysis, the characterisation of metabolites still faces considerable challenges [71]. Thus, to solve these analytical difficulties, plant metabolomics methods and equipment are being developed quickly.

2.4.3 Metabolomics: challenges and prospects in abiotic stress

Combination of metabolomics and genetic approaches, researchers can gain a better understanding of plant genetic regulation in connection to metabolomics [40]. However, there are only a few thousand metabolites that can be measured. Contrary to high throughput methods for protein, RNA, and DNA analysis, the characterisation of metabolites still faces considerable challenges. Furthermore, time can be saved by combining high-throughput genome sequencing and reverse genetics with metabolomics approaches, as in metabolomics-assisted breeding. As a result, these revolutionary plant-breeding approaches can help agricultural development programmes produce high-yielding crops, stress-tolerant germplasm, and climate-adapted crop varieties [73]. Prospects for metabolomics include investigating metabolic indicators to better understand plant metabolism [39]. Single-cell metabolomics with metabolome-scale labelling, for example, will improve single-cell metabolite interpretation, metabolic pathway elucidation, and metabolite quantification. The future prospects for metabolomics will be to better exploit the available information from metabolomics and to understand the metabolite information for prospective applications.

2.5 Phonemics

Phenotype is defined as an observable biophysical trait of an organism, such as its appearance, behaviour, and development [74]. The high-throughput phenotyping analysis is employed in the field of phenomics. The reliability with which a genetic marker and phenotype are linked impacts genomic performance [27]. As a result, phenomics, together with a few other omics methods, offers the highest potential for plant breeding. Plant breeders use omics techniques to improve genetics to develop a perfect phenotype that provides a greater and more consistent yield under a wide range of environmental variables [16]. Recently, research that uses phenomic descriptors to aid characterisation techniques has been published [11]. Proximal sensors such as cameras, fluorometers, trichromatic, multispectral, and hyperspectral sensors are used to collect agronomic, morphological, physiological, and colourimetric data of accessions.

2.5.1 Phenotypic responses to abiotic stresses

Visible symptoms of drought tolerance in plants include leaf rolling, stay-green ability, stomatal closure, photochemical quenching, photo inhibition resistance, water use efficiency, osmotic adjustment, membrane stability, mobilisation of water-soluble carbohydrates, and increased root length. Under drought stress, these features are frequently targeted for phenotyping. The principal visible sign and one of the survival mechanisms against drought stress are leaf rolling, which reduces transpiration rate and canopy temperature [72].

Osmotic stress diminishes root system water absorption capacity while increasing leaf water loss [75]. Membrane disruption, nutritional imbalance, the diminished ability of ROS detoxification, variations in antioxidant enzymes, lower photosynthetic activity, and reduced stomatal aperture are all key physiological alterations caused by osmotic stress. Ion toxicity is caused primarily by increased accumulation of Na^+ and Cl^- ions in plant tissues exposed to high saline environments. Increased Na^+ and Cl^- absorption into cells causes a severe ionic imbalance and may induce significant physiological effects. Phenopsis and other next-generation phenotyping tests that use different light wavelengths are used to assess salt tolerance [76].

Heat stress changes phenotypic and biochemical aspects in cultivated plant species. Such as low germination ratio, poor seedling emergence, aberrant seedling development, poor seedling vigour, and reduced radicle and plumule growth [77]. Even a few degrees of temperature increase during flowering can result in total yield loss.

Low temperatures harm plant growth and productivity, resulting in large output losses. Cold stress is classified as chilling stress (biomembrane lipid composition and the accumulation of small molecules) based on the temperature range. Tropical and subtropical plants are more vulnerable to chilling stress and lack the cold adaptation mechanism [72].

2.5.2 Phenomics tools

Manual phenotyping, which takes time and is usually subjective, may cause the development of high-yielding crop varieties matched to a certain area to be delayed. High-throughput, non-destructive field crop evaluation and controlled circumstances are lacking in our breeding systems. The necessity for plant phenomics infrastructure and methodology is now generally accepted [72]. For instance, Infrared thermography, Spectroscopic techniques, Raman spectroscopy, and Fluorescence imaging approaches can be used to explore various phenotypic rates.

2.5.3 Phenomics: challenges and prospects

Phenomics data collection is difficult, demanding the collaboration of experts from diverse fields, costly, and time-consuming. Screen plants for desirable, repeated trials in a variety of conditions are required. Individual plant measurement in controlled conditions has been the subject of much phenotyping controversy; plant development in the open environment is not accurately represented in a controlled environment. Taking all of this into account, the primary issues have been identified as a considerable gap in plant performance from lab to field. Even though field phenotyping is a practical requirement in crop, high-throughput field phenotyping lags behind current indoor phenotypic facilities. To increase their accuracy, Artificial Intelligence (AI) technologies require a significant amount of data from various sources [78]. The use of already available AI-adaptable technology, like smartphones, to boost the number and quality of data collected [79].

2.6 Ionomics

Ionomics is the study of the complete ionome of a tissue/an organism, involving the quantification of all elemental constituents in reaction to physiological processes or changes [46]. The concept of an 'ionome' was first defined as the metals, nonmetals, and metalloids present in an organism, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulphur (S), and magnesium (Mg) play a part in the ionome, as do trace metals such as iron (Fe), copper, and zinc (Cu),

manganese (Mn), molybdenum (Mo), cobalt (Co), and zinc (Zn) in heavy metal toxicity reduction. Later, the term 'ionome' was extended to refer to a collection of biologically important non-metals, such as N, P, and S [80].

2.6.1 Ionomics for abiotic stress resilience

Salinity stress increases Na^+ uptake while decreasing K^+, $Ca^{2+,}$ Mg^{+2}, and NO^{-3} absorption [81]. Cd tolerant cultivars accumulated much more proline, phenolics, and antioxidants, as well as increased rhizosphere and root cell sap absorption and translocation of N, P, K, $Ca^{2+,}$ Mg, Zn, and Fe. These nutrients are essential for plants to respond to stresses by increasing a variety of physiological, biochemical, and molecular responses [82].

Toxicity to Na^+ and F has been reported to suppress growth and yield in *M. halliana* and rice, respectively. However, this problem was overcomed by increasing the concentrations of Ca^{2+}-mediated signalling for maintaining Na^+/K^+ homeostasis, as well as reducing stress-induced injury for Na^+ toxicity, whereas increases the levels of essential co-factors, namely, Fe, Zn, and Cu, which further enhance the activity of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase [83].

2.6.2 Gene/quantitative trait loci for ionomics

Ionomics analysis of a varied range of genotypes using molecular marker data allows for the rapid identification of genomic loci influencing a trait [84]. Many QTL mapping studies have been conducted to identify loci/genes related to a wide range of ionomic traits. Numerous natural alleles that regulate ionomic traits have been found using the GWAS approach [82].

2.6.3 Ionomics analysis

In the recent decade, ionomics techniques are classified into two classes depending on atom electronic characteristics (emission, absorption, and fluorescence spectroscopy) and nuclear properties (radioactivity or atomic number) [85]. High-throughput analytical techniques such as liquid chromatography coupled to photodiode array/mass spectrometry (LC-PDA/MS), plasma optical emission spectroscopy (ICP-OES), liquid chromatography coupled to mass spectrometry (LC-MS), X-ray fluorescence (XRF), capillary electrophoresis coupled to mass spectrometry (CE-MS), gas chromatography coupled to mass spectrometry (GC-MS), nuclear magnetic resonance (NMR) spectroscopy, and Fourier transformtion cyclotron resonance mass spectrometry (FT-ICR/MS) are sufficient and suitable for application in ionomics studies of plants [82].

3. Conclusion

In many countries, faba beans are still one of the most important staple foods and feed legume crops, supporting smallholder farmers in raising their level of living. Abiotic stress is harmful to faba bean growth and development. Faba beans are susceptible to salinity, droughts, frost, waterlogging, heavy metals, and temperature swings. Plant morphological, physiological, and biochemical parameters are all affected by abiotic stress. Omics is a rapidly expanding technique that allows researchers to better comprehend and detect all genomics, transcriptomics, proteomics, metabolomics, phenomics, and ionomics events that occur in plants as a result of abiotic stress. SNP markers from faba bean were used to identify 67 putative QTL for physiological and morphological characteristics associated with frost tolerance. Furthermore, a large number of transcription factor genes have been identified as being involved in the modulation of stress signal transduction pathways in response to drought, cold, or excess salt. These faba bean transcription factors may establish gene networks and regulate the expression of stress-inducible genes. The proteomics is critical for comprehending the principles that govern how cells function and respond to various stimuli. Depending on the genotype and the level of tolerance studied, plants may accumulate or increase the production of certain proteins with defensive effects. Metabolomics is essential for comprehending the chemical signals emitted by plants as they grow and thrive. Metabolomics revealed that enhanced proline and soluble sugar buildup in faba beans improved drought tolerance. Phonemics is a complicated interplay of heredity, phenotype, and environment. Ionomic is used to better understand the mechanism of mineral transport in plants by identifying and testing putative transporter genes for activity of faba bean in a timely and cost-effective manner.

Author Contributions

Conceptualization, B.M; writing—original draft preparation, B.M., A.A, D.M., and T.Z; review and editing. All authors have read and agreed on the version of the final draft of this manuscript for submission and have given consent for the publication of identifiable details.

References

[1] Caracuta V, Weinstein-Evron M, Kaufman D, Yeshurun R, Silvent J, Boaretto E. 14,000-year-old seeds indicate the Levantine origin of the lost progenitor of faba bean. Sci Rep. 2016;6(1):37399.

[2] Alharbi NH, Adhikari KN. Factors of yield determination in faba bean (Vicia faba). Crop Pasture Sci. 2020;71(4):305-21.

[3] Khazaei H, O'Sullivan DM, Stoddard FL, et al. Recent advances in faba bean genetic and genomic tools for crop improvement. Legume Sci. 2021;3(3):e75.

[4] Warsame AO, O'Sullivan DM, Tosi P. Seed storage proteins of faba bean (Vicia faba L): Current status and prospects for genetic improvement. J Agric Food Chem. 2018;66(48):12617-26.

[5] Abou-Khater L, Maalouf F, Rubiales D. Status of faba bean (Vicia faba L.) in the Mediterranean and East African countries. In: Developing Climate Resilient Grain and Forage Legumes. Singapore: Springer Nature Singapore; 2022. p. 297-327.

[6] Abdelhaleim MS, Rahimi M, Okasha SA. Assessment of drought tolerance indices in faba bean genotypes under different irrigation regimes. Open Life Sci. 2022;17(1):1462-72.

[7] Shah WH, Rasool A, Saleem S, et al. Understanding the integrated pathways and mechanisms of transporters, protein kinases, and transcription factors in plants under salt stress. Int J Genomics. 2021;2021.

[8] Kosová K, Vítámvás P, Urban MO, Prášil IT, Renaut J. Plant abiotic stress proteomics: the major factors determining alterations in cellular proteome. Front Plant Sci. 2018;9:122.

[9] Maalouf F. Developing improved varieties of faba bean. Burleigh Dodds Sci. 2018.

[10] Mansour E, Desoky ESM, Ali MM, et al. Identifying drought-tolerant genotypes of faba bean and their agro-physiological responses to different water regimes in an arid Mediterranean environment. Agric Water Manag. 2021;247:106754.

[11] Muktadir MA, Adhikari KN, Merchant A, et al. Physiological and biochemical basis of faba bean breeding for drought adaptation—A review. Agronomy. 2020;10(9):1345.

[12] Maalouf F, Hu J, O'Sullivan DM, et al. Breeding and genomics status in faba bean (Vicia faba). Plant Breed. 2019;138(4):465-73.

[13] Gutiérrez N, Pégard M, Balko C, Torres AM. Genome-wide association analysis for drought tolerance and associated traits in faba bean (Vicia faba L.). Front Plant Sci. 2023;14.

[14] Awaad HA, Awaad HA. Approaches in faba bean to mitigate impact of climate change. In: Sustainable Agriculture in Egypt: Climate Change Mitigation. 2022. p. 185-217.

[15] Jan N, Rather AMUD, John R, et al. Proteomics for abiotic stresses in legumes: present status and future directions. Crit Rev Biotechnol. 2023;43(2):171-90.

[16] Chen CJ, Rutkoski J, Schnable JC, et al. Role of the genomics–phenomics–agronomy paradigm in plant breeding. Plant Breed Rev. 2022;46:627-73.

[17] Afzal M, Alghamdi SS, Migdadi HH, et al. Legume genomics and transcriptomics: From classic breeding to modern technologies. Saudi J Biol Sci. 2020;27(1):543-55.

[18] Ali A, Altaf MT, Nadeem MA, et al. Recent advancement in OMICS approaches to enhance abiotic stress tolerance in legumes. Front Plant Sci. 2022;13.

[19] Jha UC, Bohra A, Nayyar H. Advances in "omics" approaches to tackle drought stress in grain legumes. Plant Breed. 2020;139(1):1-27.

[20] Ziegler G, Nelson R, Granada S, et al. Genomewide association study of ionomic traits on diverse soybean populations from germplasm collections. Plant Direct. 2018;2(1):e00033.

[21] Mathivanan S. Abiotic Stress-Induced Molecular and Physiological Changes and Adaptive Mechanisms in Plants. In: Abiotic Stress in Plants. 2021. p. 315.

[22] Naik B, Kumar V, Rizwanuddin S, et al. Genomics, Proteomics, and Metabolomics Approaches to Improve Abiotic Stress Tolerance in Tomato Plant. Int J Mol Sci. 2023;24(3):3025.

[23] Kumar P, Choudhary M, Halder T, et al. Salinity stress tolerance and omics approaches: Revisiting the progress and achievements in major cereal crops. Heredity. 2022;128(6):497-518.

[24] Muthamilarasan M, Singh NK, Prasad M. Multi-omics approaches for strategic improvement of stress tolerance in underutilized crop species: a climate change perspective. Adv Genet. 2019;103:1-38.

[25] Sharma V, Gupta P, Kagolla P, et al. Metabolomics intervention towards better understanding of plant traits. Cells. 2021;10(2):346.

[26] Parida AK, Panda A, Rangani J. Metabolomics-guided elucidation of abiotic stress tolerance mechanisms in plants. In: Plant Metabolites and Regulation under Environmental Stress. Academic Press; 2018. p. 89-131.

[27] Varshney RK, Thudi M, Pandey MK, et al. Accelerating genetic gains in legumes for the development of prosperous smallholder

agriculture: integrating genomics, phenotyping, systems modelling and agronomy. J Exp Bot. 2018;69(13):3293-3312.

[28] Carrillo-Perdomo E, Vidal A, Kreplak J, et al. Development of new genetic resources for faba bean (Vicia faba L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map. Sci Rep. 2020;10(1):6790.

[29] O'Sullivan DM, Angra D, Harvie T, Tagkouli V, Warsame A. A genetic toolbox for Vicia faba improvement. In: International Conference on Legume Genetics and Genomics. 2019.

[30] Mandour H, Khazaei H, Stoddard FL, Dodd IC. Identifying physiological and genetic determinants of faba bean transpiration response to evaporative demand. Ann Bot. 2023.

[31] Mokhtar MM, Hussein EH, El-Assal SEDS, Atia MA. Vf ODB: a comprehensive database of ESTs, EST-SSRs, mtSSRs, microRNA-target markers and genetic maps in Vicia faba. AoB Plants. 2020;12(6):plaa064.

[32] Webb A, Cottage A, Wood T, et al. A SNP-based consensus genetic map for synteny-based trait targeting in faba bean (Vicia faba L.). Plant Biotechnol J. 2016;14(1):177-85.

[33] Khan MA, Alghamdi SS, Ammar MH, et al. Transcriptome profiling of faba bean (Vicia faba L.) drought-tolerant variety hassawi-2 under drought stress using RNA sequencing. Electron J Biotechnol. 2019;39:15-29.

[34] Sallam A, Arbaoui M, El-Esawi M, Abshire N, Martsch R. Identification and verification of QTL associated with frost tolerance using linkage mapping and GWAS in winter faba bean. Front Plant Sci. 2016;7:1098.

[35] Siddiqui MH, Al-Khaishany MY, Al-Qutami MA, et al. Response of different genotypes of faba bean plant to drought stress. Int J Mol Sci. 2015;16(5):10214-27.

[36] Ewas M, Omar SA, El-Sarag E, Mubarak MH. Genome-Wide Characterization of Drought-Responsive Genes Accelerates Faba Bean Breeding Program. Egypt J Desert Res. 2021;69(2):47-67.

[37] Lyu JI, Ramekar R, Kim JM, et al. Unraveling the complexity of faba bean (Vicia faba L.) transcriptome to reveal cold-stress-responsive genes using long-read isoform sequencing technology. Sci Rep. 2021;11(1):21094.

[38] Biswas D, Saha SC, Dey A. CRISPR-Cas genome-editing tool in plant abiotic stress-tolerance. Plant Gene. 2021;26:100286.

[39] Kaur B, Sandhu KS, Kamal R, et al. Omics for the improvement of abiotic, biotic, and agronomic traits in major cereal crops: Applications, challenges, and prospects. Plants. 2021;10(10):1989.

[40] Yang Y, Saand MA, Huang L, et al. Applications of multi-omics technologies for crop improvement. Front Plant Sci. 2021;12:563953.

[41] Alghamdi SS, Khan MA, Ammar MH, et al. Characterization of drought stress-responsive root transcriptome of faba bean (Vicia faba L.) using RNA sequencing. 3 Biotech. 2018;8:1-19.

[42] Wu X, Fan Y, Li L, Liu Y. The influence of soil drought stress on the leaf transcriptome of faba bean (Vicia faba L.) in the Qinghai–Tibet Plateau. 3 Biotech. 2020;10:1-16.

[43] Braich S, Sudheesh S, Forster JW, Kaur S. Characterisation of faba bean (Vicia faba L.) transcriptome using RNA-seq: sequencing, de novo assembly, annotation, and expression analysis. Agronomy. 2017;7(3):53.

[44] Javed T, Shabbir R, Ali A, et al. Transcription factors in plant stress responses: Challenges and potential for sugarcane improvement. Plants. 2020;9(4):491.

[45] Yin X, Zhang Y, Zhang L, et al. Regulation of MYB transcription factors of anthocyanin synthesis in lily flowers. Front Plant Sci. 2021;12:2677.

[46] Singh RK, Sood P, Prasad A, Prasad M. Advances in omics technology for improving crop yield and stress resilience. Plant Breed. 2021;140(5):719-31.

[47] Yang W, Feng H, Zhang X, et al. Crop phenomics and high-throughput phenotyping: past decades, current challenges, and future perspectives. Mol Plant. 2020;13(2):187-214.

[48] Heinrich F, Wutke M, Das PP, et al. Identification of regulatory SNPs associated with vicine and convicine content of Vicia faba based on genotyping by sequencing data using deep learning. Genes. 2020;11(6):614.

[49] Gao B, Bian XC, Yang F, et al. Comprehensive transcriptome analysis of faba bean in response to vernalization. Planta. 2020;251:1-15.

[50] Björnsdotter E, Nadzieja M, Chang W, et al. VC1 catalyses a key step in the biosynthesis of vicine in faba bean. Nat Plants. 2021;7(7):923-31.

[51] Escobar-Herrera L, Kreplak J, Nadzieja M, et al. The faba bean pan-transcriptome. In: PAG XXVIII, Plant and Animal Genome. 2020.

[52] Razzaq MK, Aleem M, Mansoor S, et al. Omics and CRISPR-Cas9 approaches for molecular insight, functional gene analysis, and stress tolerance development in crops. Int J Mol Sci. 2021;22(3):1292.

[53] Li P, Zhang Y, Wu X, Liu Y. Drought stress impact on leaf proteome variations of faba bean (Vicia faba L.) in the Qinghai–Tibet Plateau of China. 3 Biotech. 2018;8:1-12.

[54] Yahoueian SH, Bihamta MR, Babaei HR, Bazargani MM. Proteomic analysis of drought stress response mechanism in soybean (Glycine

max L.) leaves. Food Sci Nutr. 2021;9(4):2010-20.

[55] Long R, Li M, Zhang T, et al. Comparative proteomic analysis reveals differential root proteins in Medicago sativa and Medicago truncatula in response to salt stress. In: The Model Legume Medicago truncatula. 2019. p. 1102-11.

[56] Ghatak A, Chaturvedi P, Bachmann G, et al. Physiological and proteomic signatures reveal mechanisms of superior drought resilience in pearl millet compared to wheat. Front Plant Sci. 2021;11:600278.

[57] Xu Y, Fu X. Reprogramming of plant central metabolism in response to abiotic stresses: a metabolomics view. Int J Mol Sci. 2022;23(10):5716.

[58] Tadele M. Impacts of soil acidity on growth performance of faba bean (Vicia faba L) and management options. Acad Res J Agric Sci Res. 2020;8(4):423-31.

[59] Labuschagne M, Masci S, Tundo S, et al. Proteomic analysis of proteins responsive to drought and low temperature stress in a hard red spring wheat cultivar. Molecules. 2020;25(6):1366.

[60] Baslam M, Mitsui T. The Future of Rice Demand: Quality Beyond Productivity. In: Proteomic for quality: Mining the proteome as a strategy to elucidate the protein complex applied for quality improvement. Springer Nature; 2020. p. 473-94.

[61] Carrera FP, Noceda C, Maridueña-Zavala MG, Cevallos-Cevallos JM. Metabolomics, a powerful tool for understanding plant abiotic stress. Agronomy. 2021;11(5):824.

[62] Alseekh S, Bermudez L, De Haro LA, Fernie AR, Carrari F. Crop metabolomics: From diagnostics to assisted breeding. Metabolomics. 2018;14:1-13.

[63] Abdelrahman M, Burritt DJ, Tran LSP. The use of metabolomic quantitative trait locus mapping and osmotic adjustment traits for the improvement of crop yields under environmental stresses. Semin Cell Dev Biol. 2018;83:86-94.

[64] Dawid C, Hille K. Functional metabolomics—a useful tool to characterize stress-induced metabolome alterations opening new avenues towards tailoring food crop quality. Agronomy. 2018;8(8):138.

[65] Migdadi HM, El-Harty EH, Salamh A, Khan MA. Yield and proline content of faba bean genotypes under water stress treatments. JAPS. 2016;26(6).

[66] Bista DR, Heckathorn SA, Jayawardena DM, Mishra S, Boldt JK. Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and-tolerant grasses. Plants. 2018;7(2):28.

[67] Belachew KY, Stoddard FL. Screening of faba bean (Vicia faba L.) accessions to acidity and aluminium stresses. PeerJ. 2017;5:2963.

[68] Wang X, Guo R, Li M, et al. Metabolomics reveals the drought-tolerance mechanism in wild soybean (Glycine soja). Acta Physiol Plant. 2019;41:1-11.

[69] Ghatak A, Chaturvedi P, Weckwerth W. Metabolomics in plant stress physiology. In: Plant Genetics and Molecular Biology. 2018. p. 187-236.

[70] Richter JA, Behr JH, Erban A, Kopka J, Zörb C. Ion-dependent metabolic responses of Vicia faba L. to salt stress. Plant Cell Environ. 2019;42(1):295-309.

[71] Bueno PC, Lopes NP. Metabolomics to characterize adaptive and signaling responses in legume crops under abiotic stresses. ACS Omega. 2020;5(4):1752-63.

[72] Singh B, Mishra S, Bohra A, et al. Crop phenomics for abiotic stress tolerance in crop plants. In: Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants. 2018. p. 277-96.

[73] Scossa F, Alseekh S, Fernie AR. Integrating multi-omics data for crop improvement. J Plant Physiol. 2021;257:153352.

[74] Pratap A, Gupta S, Nair RM, et al. Using plant phenomics to exploit the gains of genomics. Agronomy. 2019;9(3):126.

[75] Sandhu D, Kaundal A. Dynamics of salt tolerance: molecular perspectives. In: Biotechnologies of Crop Improvement, Volume 3: Genomic Approaches. 2018. p. 25-40.

[76] Jangra S, Chaudhary V, Yadav RC, Yadav NR. High-throughput phenotyping: a platform to accelerate crop improvement. Phenomics. 2021;1(2):31-53.

[77] Hassan MU, Chattha MU, Khan I, et al. Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—A review. Plant Biosyst. 2021;155(2):211-34.

[78] Jung J, Maeda M, Chang A, et al. The potential of remote sensing and artificial intelligence as tools to improve the resilience of agriculture production systems. Curr Opin Biotechnol. 2021;70:15-22.

[79] Javaid M, Haleem A, Khan IH, Suman R. Understanding the potential applications of Artificial Intelligence in Agriculture Sector. Adv Agrochem. 2023;2(1):15-30.

[80] Chen A, Hansen TH, Olsen LI, et al. Towards single-cell ionomics: a novel micro-scaled method for multi-element analysis of nanogram-sized biological samples. Plant Methods. 2020;16(1):1-13.

[81] Serafin-Andrzejewska M, Jama-Rodzeńska A, Helios W, Kotecki A, Kozak M, Białkowska M, Bárta J, Bártová V. Accumulation of Minerals in Faba Bean Seeds and Straw in Relation to Sowing Density. Agriculture. 2023;13(1):147.

[82] Ali S, Tyagi A, Bae H. Ionomic approaches for discovery of novel stress-resilient genes in plants. Int J Mol Sci. 2021;22(13):7182.

[83] Banerjee A, Singh A, Sudarshan M, Roychoudhury A. Silicon nanoparticle-pulsing mitigates fluoride stress in rice by fine-tuning the ionomic and metabolomic balance and refining agronomic traits. Chemosphere. 2021;262:127826.

[84] Nazir M, Mahajan R, Mansoor S, Rasool S, Mir RA, Singh R, Zargar SM. Identification of QTLs/Candidate Genes for Seed Mineral Contents in Common Bean (Phaseolus vulgaris L.) Through Genotyping-by-Sequencing. Front Genet. 2022;13:143.

[85] Mukherjee R, Barwant M, Sinha D. Ionomics vis à vis Heavy Metals Stress and Amelioration. In: Heavy Metals in Plants Physiological to Molecular Approach. 2022:246-280.